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Vascular Reactivity Is Impaired in Genetic Females Taking High-Dose Androgens

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Objective. **To assess the vascular effects of high-dose androgen treatment in genetic females.**

Background. **Male gender is an independent risk factor for coronary artery disease, suggesting either a protective effect of estrogens and/or a deleterious effect of androgens. We have recently demonstrated that androgen deprivation is associated with enhanced vascular reactivity in adult men, however, the effects of androgen excess on vascular function in humans has not been reported previously.**

Methods. **We studied vascular reactivity in two groups of genetic females: 12 female-to-male transsexuals receiving long-term high-dose androgens, and 12 healthy female control subjects, matched for age and smoking history. Using external vascular ultrasound, brachial artery diameter was measured at rest, after flow increase (leading to flow-mediated dilatation [FMD], which depends on normal endothelial function) and after sublingual nitroglycerin (NTG), an endothelium-independent dilator.**

Results. **Testosterone levels were higher (15.2 ± 8.7 vs. 1.9 ± 1.3 mmol/L, $p < 0.001$) and high-density lipoprotein cholesterol levels were lower (1.2 ± 0.2 vs. 1.6 ± 0.4 mmol/L, $p = 0.02$) in the transsexuals compared with the control subjects. In each group, nine of 12 subjects were current or ex-smokers, leading to impaired FMD in both groups (5.1 ± 3.7% in the transsexuals vs. 6.9 ± 4.1% in controls, $p = 0.28$). The NTG response was significantly decreased in the transsexuals (15.9 ± 4.9% vs. 22 ± 5.8% in controls, $p = 0.01$), independent of the effects of age, cholesterol or vessel size.**

Conclusions. **Long-term treatment with high-dose androgens is associated with impaired vascular reactivity in genetic females, consistent with a deleterious effect of androgen excess on arterial physiology.**

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An important gender difference exists in the incidence of cardiovascular disease, with males being at higher risk than females (1). Many epidemiologic and experimental studies have addressed the possible protective effects of estrogens, including lipid-lowering, antioxidant and direct arterial wall benefits (2–4).

Less well tested, however, is the possibility that androgens may have a detrimental effect on the vessel wall. In recent work, Adams et al. (5) have found that testosterone administration increases atheroma in cholesterol-fed female monkeys, and Hutchison et al. (6) have demonstrated androgen-induced endothelial dysfunction in a rabbit model of atherosclerosis. In humans, we have recently found that androgen deprivation by medical and/or surgical castration is associated with enhanced vascular reactivity in adult men (7). States of androgen excess, however, are relatively rare in humans, and to date no studies on the effects of high-dose androgens on vascular function have been reported.

Impaired vascular reactivity is an important early event in atherogenesis (8) and may determine dynamic plaque behavior in patients with advanced coronary artery disease (9). We have therefore studied endothelium and smooth muscle–dependent vascular responses in a relatively uncommon group of subjects treated with long-term, high-dose androgen therapy; that is, female-to-male transsexual adults.

Methods

Subjects. We investigated 12 consecutively recruited female-to-male transsexuals aged 33 ± 5 (range 26 to 46) years, who had been receiving high-dose androgen therapy for at least 2 months. Their duration of hormonal therapy was 38 ± 52 (range 2 to 177) months. For each transsexual subject, a healthy female control was also investigated; age and active tobacco smoking history were carefully matched between the groups, owing to the known influence of these factors on vascular reactivity (10,11). The control subjects were aged 31 ± 7 (range 26 to 41) years. In two cases where subjects could be

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Abbreviations and Acronyms

FMD = flow-mediated dilatation
HDL = high-density lipoprotein
NTG = nitroglycerin

studied before and after commencing androgen therapy, the subjects served as their own controls; controls for the other 10 transsexuals were recruited from among hospital staff and community volunteers. No subjects had a history of clinical atherosclerosis, hypertension or diabetes mellitus, and none was taking regular cardiovascular medications.

Of the 12 transsexual subjects, 10 were treated with testosterone depot implants (200 to 800 mg) and 2 were receiving regular intramuscular testosterone ester injections. Four had had hysterectomies performed, in two cases with ovariectomy (these latter two subjects had been taking piperazine estrone sulfate 2.5 mg/day since their ovariectomy), and two other subjects had undergone gender reassignment surgery 2 to 12 years before their study visit. One had a history of schizophrenia and was taking risperidone (5 mg daily). The other 11 transsexuals were medically well, as were the control subjects. All subjects gave informed consent, and the study was approved by our institutional committee on ethical practice.

Study design. Each patient had at least one visit to the study center, during which a history was taken, supine resting blood pressure was measured and a nonfasting blood sample was taken for estimation of total and high-density lipoprotein (HDL) cholesterol (using Hitachi 747 autoanalyzer [Boehringer Mannheim, Castle Hill, New South Wales, Australia]; the HDL fraction levels after precipitation with phosphotungstate-magnesium), and total and free testosterone levels (using radioimmunoassay).

Arterial reactivity was measured in the right brachial artery using external vascular ultrasound, as described in detail elsewhere (7,10). In brief, flow-mediated dilatation (FMD) was measured as the change in arterial diameter during a condition of reactive hyperemia, and smooth muscle–dependent response was measured as the change in diameter after a 400-μg spray of sublingual nitroglycerin (NTG). The accuracy and reproducibility of this method have previously been established, with a coefficient of variation for measurement of FMD in our laboratory of approximately 2% (8,12). The degree of hyperemia is calculated from Doppler-derived flow measurements during the condition of reactive hyperemia, compared with rest flow (11). Flow-mediated dilatation measured in this way is endothelium dependent, as it is predominantly due to nitric oxide release by the endothelium (13). This response in the brachial artery correlates well with coronary endothelial function (14). The NTG-induced vasodilatation is endothelium independent and reflects smooth muscle function, being mediated via the activation of guanylate cyclase and consequent increase in intracellular cyclic GMP. Ultrasound analysis was performed in each case by two independent observers who had no knowledge of each subject's identity or the stage of each series of scans, as reported previously (12).

Table 1. Baseline Characteristics and Vascular Study Results in Female-to-Male Transsexuals (n = 12) and Genetic Female Control Subjects (n = 12)

Characteristic	Transsexuals	Controls	p Value*
Age (yr)	33 ± 6	31 ± 6	0.80
Smoking†	6/3/3	6/3/3	—
Testosterone (nmol/L)‡	15.2 ± 8.7	1.9 ± 1.3	< 0.001
Free testosterone (pmol/L)‡	225 ± 145	38 ± 29	< 0.001
Total cholesterol (mmol/L)	5.2 ± 0.9	5.1 ± 1.0	0.80
HDL cholesterol (mmol/L)	1.2 ± 0.2	1.6 ± 0.4	0.02
Baseline flow (mL/min)	47 ± 22	40 ± 31	0.80
Vessel size (mm)	3.8 ± 0.3	3.1 ± 0.4	< 0.001
SBP (mm Hg)	117 ± 12	124 ± 17	0.64
DBP (mm Hg)	74 ± 11	73 ± 9	0.80
FMD (%)	5.1 ± 3.7	6.9 ± 4.1	0.28
NTG (%)	15.9 ± 4.9	22 ± 5.8	0.01
Hyperemia (%)	571 ± 280	674 ± 422	0.80

Data are presented as mean ± SD. DBP = diastolic blood pressure; FMD = flow-mediated dilatation; NTG = nitroglycerin-induced dilatation; HDL = high density lipoprotein; SBP = systolic blood pressure. *p Values adjusted for multiple comparisons (see Methods). †Smoking history; current/former/never smoker. ‡Normal ranges for healthy male adults are 11–35 nmol/L for total and 170–510 pmol/L for free testosterone.

Statistics. Descriptive data are expressed as mean value ± SD. The two groups of subjects (transsexuals and controls) were compared by independent samples *t* tests. The prospectively defined primary end points of this study were FMD and NTG responses. All other *t* test results were adjusted for multiple comparisons using Hochberg's modification of the Bonferroni procedure (15). The determinants of FMD and NTG-induced dilatation were assessed by univariate and multivariate linear regression analyses with age, vessel size, total or HDL cholesterol and androgen therapy being entered as the independent variables. Statistical significance was inferred at a two-sided p value <0.05.

Results

Baseline characteristics. The female-to-male transsexuals and control subjects were well matched for age, smoking history, total cholesterol level, blood pressure and resting blood flow (Table 1). The average pack/year cigarette consumption in the current and former smokers was similar in each group; 13 ± 8 for the transsexuals and 13 ± 7 for control subjects. In the androgen-treated genetic females, HDL cholesterol was lower, vessel size was larger and testosterone levels were higher. In the transsexual subjects, serum estradiol levels were 144 ± 39 pmol/L (normal range for premenopausal women, 75 to 1300 pmol/L), progesterone levels were 1.04 ± 0.2 nmol/L (normal range, <2 to 90 nmol/L), follicle stimulating hormone levels were 15.3 ± 18.6 IU/L (normal range, 1.5 to 20 IU/L) and sex hormone binding globulin levels were 29.9 ± 9.6 nmol/L (normal range, 15 to 140 nmol/L).

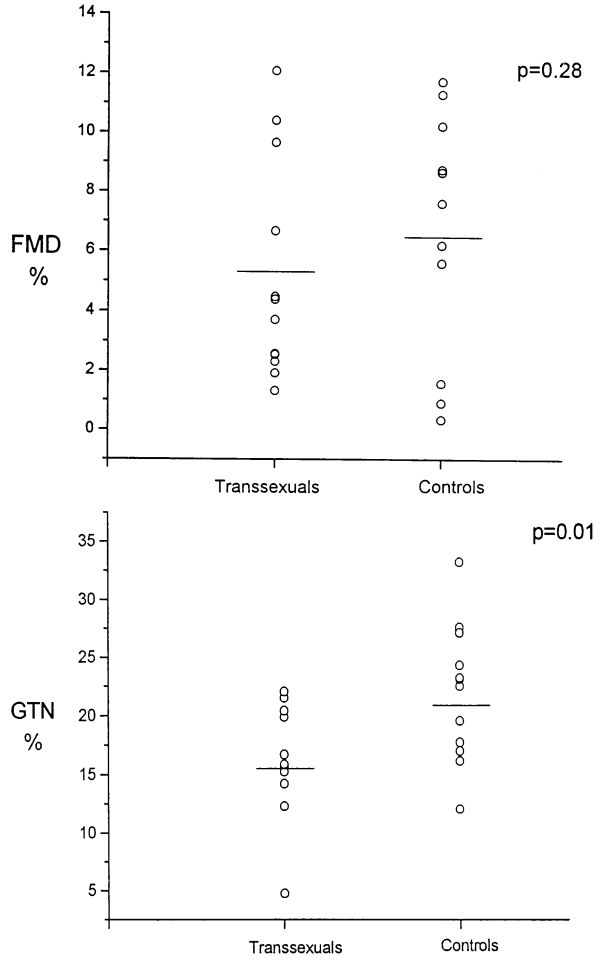

Figure 1. Flow-mediated and nitroglycerin-induced dilatation in 12 genetic females receiving high-dose androgens (female-to-male transsexuals) and in 12 female control subjects. The *y* axes refer to percent change in brachial artery diameter. GTN = glyceryl trinitrate (nitroglycerin).

Vascular reactivity. The degree of reactive hyperemia after cuff deflation (the stimulus to FMD) was >500% in both groups. In response to this flow increase, FMD was 5.1 ± 3.7% in the female-to-male transsexuals and 6.9 ± 4.1% in the control subjects ($p = 0.28$) (Fig. 1). In contrast, FMD measured by the same technique in healthy young female nonsmokers is approximately 10% (9,10). The NTG response, unaffected by cigarette smoking (8), was significantly impaired in the transsexuals (15.9 ± 4.9% vs. 22 ± 5.8% in the controls; $p = 0.01$), consistent with decreased smooth muscle dilator function. There was no significant correlation between NTG response and the recorded blood pressure ($p > 0.3$). In the two subjects studied before and after androgen therapy was commenced, NTG-induced dilatation decreased from 25% to 18% in one, and 22% to 16% in the other, after 2 to 4 months of hormone treatment. In the same subjects, FMD declined from 11% to 2% in one, but was unchanged in the other (a heavy ex-smoker).

On multivariate analysis, FMD was not related to androgen therapy, vessel size, cholesterol or testosterone levels. The NTG response, however, was significantly impaired in the transsexuals (partial correlation coefficient = −0.65, $p = 0.03$) independent of the effects of age, vessel size or total cholesterol levels. In this multivariate model, the multiple r value was 0.80, the F value was 4.32 and the significance of F was 0.02. When HDL cholesterol rather than total cholesterol was entered into the multivariate model, HDL levels were not independently related to the NTG response.

Discussion

The greater predisposition of males to coronary artery disease has focused recent attention on the role of sex hormones in atherogenesis. These steroids may have complex vascular effects, including those on arterial structure and function, as well as on coagulation pathways and vasoactive factors. Indeed, many studies have suggested that estrogens may be atheroprotective agents, via lipid-lowering, antioxidant and/or direct vascular function effects (16). In contrast, relatively little has been written about the vascular effects of androgens in human females or males. In this study, we report the effects of long-term high-dose androgens in humans for the first time in an uncommon group of subjects; female-to-male transsexuals. Although the group is small and the measurement of endothelial function confounded by a high proportion of smokers, vascular reactivity was impaired in the transsexual subjects, consistent with a deleterious effect of androgen excess on arterial physiology.

Previous studies: androgens and vascular effects. In acute infusion studies, testosterone in supraphysiologic doses appears to act as a direct vasodilator and may be associated with relief of angina pectoris in men (17), possibly via K^+ channel–related coronary vasorelaxant effects (18). The more chronic effects of androgens, however, have only been examined in a variety of animal models, with often conflicting results (19–21).

The long-term vascular effects of androgens in humans have been difficult to study, in part because there is no clinical syndrome or phenotype for androgen excess in men. By contrast, estrogen supplementation (postmenopausal women taking hormone replacement) and deficiency (premature menopause) have been much more common and therefore more amenable to investigation. Recently, we studied a group of men with long-term complete androgen deprivation as therapy for prostate cancer; in these men, medical and/or surgical castration was associated with enhanced vascular reactivity, consistent with a deleterious effect of normal male levels of testosterone on the vessel wall (7). Furthermore, we and others have found vascular reactivity to be enhanced in male-to-female transsexuals who were taking high-dose estrogens (22,23). This latter observation may be due to the effects of the estrogen excess and/or the androgen deprivation due to such treatment. Recently, Birdsall et al. (24) have demonstrated more severe and extensive coronary artery disease in women with polycystic ovarian syndrome compared with controls, however, this condition is characterized by other poten-

tially pro-atherogenic metabolic abnormalities (e.g., insulin resistance, obesity), in addition to mild hyperandrogenism. In the current study, we have examined some of the vascular effects of androgen excess per se, particularly finding a decrease in smooth muscle–dependent vasodilator response in two subjects studied serially with a reproducible technique (11), before and after the commencement of androgen therapy.

Mechanism. The mechanism whereby androgens might lead to impaired vascular reactivity is unknown. In some studies, testosterone therapy has been shown to decrease HDL cholesterol (25), as was observed in our current study. Overall, however, the data concerning androgens and lipoprotein profiles have not consistently shown any deleterious effect (26). It is possible that there are direct effects of androgen on the vessel wall, as steroid receptors exist in the vasculature (27). The mechanism whereby androgens may be associated with larger arterial size in genetic females is also uncertain, however, testosterone's activity as a direct smooth muscle vasorelaxant (18) may be important in this regard. In the current study, the impairment in vascular reactivity observed in the transsexual subjects was independent of the effects of vessel size.

Epidemiologic data. Many studies have confirmed the increased prevalence of premature atherosclerotic disease in men (1,28). Within each gender, however, there has been no consistent association between androgen levels within the sex-specific physiologic ranges and cardiovascular event rates. Although most cross-sectional studies suggest an inverse correlation between androgen levels and cardiovascular event rates in men, in prospective studies there has been no significant correlation demonstrated between testosterone levels and coronary disease in men (29) or between androgen levels and arterial disease in women (30). Therefore, the large difference in androgen levels between genders accounts for most of the gender difference in cardiovascular risk, and the small differences that exist within normal populations of men or women are of relatively minor significance.

Study limitations. A limitation of the current study is its cross-sectional and nonrandomized design. Although these relatively small groups should have been similar, as subjects were closely matched for age, smoking status, blood pressure and absence of other known vascular risk factors, it is still possible that unmeasured differences between groups (other than their hormonal status) were present (e.g., passive smoke exposure or homocysteine levels). A prospective study of the effects of long-term androgen therapy in women would be difficult, however, due to the limited access to transsexuals who have not already received some treatment and to the sensitivities of recruiting and investigating subjects during their period of gender transition. Two of our 12 subjects were studied serially and showed results similar to those of the overall group. Furthermore, the high prevalence of smoking in the transsexual subjects (also noted previously by Van Kesteren et al. [31]) importantly reduced the power of the study to detect any significant difference in FMD between groups, as smoking (current and former) is a major cause of endothelial dysfunction (8,32).

As impaired vascular reactivity is important both in early atherogenesis and determining plaque behavior late in the natural history of atherosclerosis (7,8), our observations of decreased arterial vasodilator responses might have adverse prognostic implications for genetic females taking androgens, however, long-term clinical follow-up of a large cohort of such female-to-male transsexual subjects would be required to assess this possibility.

Conclusion. Long-term therapy with high-dose androgens is associated with impaired vascular reactivity in genetic females, independent of the effects of androgens on lipoprotein levels or vessel size. Although interactions between sex steroids and the vessel wall are complex, these data are consistent with a deleterious effect of androgen excess in genetic females.

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